CyVerse Documentation

Release 1.0

CyVerse

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Goal

Perform RNAseq differential expression analysis using Read Mapping and Transcript Assembly (RMTA) and Rstudio-DESEq2 apps

- RMTA is a workflow that can rapidly process raw RNA-seq illumina data by mapping reads to a genome (HISAT2), assemble transcripts using Stringtie and count reads using featureCounts.
- DESeq2 is a R package to analyze count tables for differentially expressed genes, visualize the results, add extra gene annotations, and cluster samples and genes using transformed counts

In this quick start, we will show you how to launch RMTA app and Rstudio-DESeq2 VICE app in DE

4 Chapter 1. Goal

CHAPTER 2

Prerequisites

2.1 Downloads, access, and services

In order to complete this tutorial you will need access to the following services/software

Prerequisite	Preparation/Notes	Link/Download
CyVerse account	You will need a CyVerse account to complete this exercise	Register

2.2 Platform(s)

We will use the following CyVerse platform(s):

Platform	Interface	Link	Platform Documen- tation	Learning Center Docs
Discovery Environ-	Web/Point-and-	Discovery Environ-	DE Manual	Guide
ment	click	ment		

2.3 Test data for webinar

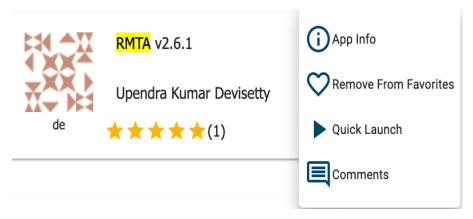
Test Case Transcriptomic profiling of Arabidopsis during sequential Drought vs Control biotic and abiotic stressses					
TO THE WAR	SRA#	Tissue_Source	Sample_Name	Library:	
	SRR3371313	Leaf	Control_7days	Name: T3CR1PP	
条件祭 华华东	SRR3371314	Leaf	Control_7days	Instrument: Illumina HiSeq 2000	
WAR THE	SRR3371315	Leaf	Control_7days	Strategy: RNA-Seq	
	SRR3371316	Leaf	Drought_7days	Source: TRANSCRIPTOMIC	
(大汉) (1)	SRR3371317	Leaf	Drought_7days	Selection: unspecified	
INT. The	SRR3371318	Leaf	Drought_7days	Layout: SINGLE	

CHAPTER 3

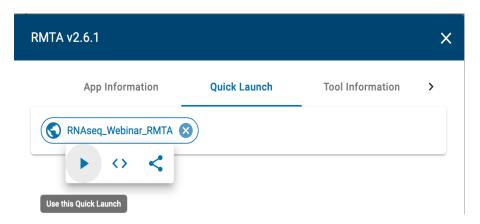
Launch RMTA_v2.6.1 with test data

Right-Click the below button and open in a new tab for quick launch of RMTA-2.6.1 analysis with test data

- 1. Login to the.
- 2. CLick on "Apps" tab in the Discovery Enviornment and search for "RMTA v2.6.1".
- 3. Click on the three dots button near the top-right corner of the app and click on "Quick Launch".



4. Click on the "RNAseq_Webinar_RMTA" quick launch and click on the play button to launch.



5. Under "Analysis Name" leave the defaults or make any desired notes.

Note: The quick launch feature comes pre-loaded with required files (reference genome, annotation and single-end fastq reads) for performing read mapping and transcript assembly of the test data.

- 6. Under "Resource Requirements" request resources as needed or leave for defaults
- 7. Click Launch Analysis. You will receive a notification that the job has been submitted and running.

Note: You will be notified when the analysis has finished successfully.

8. Click on the "Analyses" button to display the dashboard of your analyses. Click on your RMTA anlaysis to navigate to that analysis folder in your data store.

Note: We will need the "feature_counts.txt" file from the "Feature_counts" folder in the "RMTA_Output" folder for next differential expression analysis.

CHAPTER 4

Launch Rstudio-DESEq2 VICE app with test script

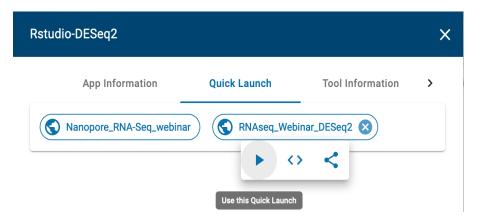
VICE is a Visual and Interactive Computing Environment which is the latest feature in CyVerse's Discovery Environment (DE) for running interactive apps such as Rstudio and Jupyter Notebooks.

Right-Click the below button and open in a new tab for quick launch of Rstudio-DESeq2 VICE app

- 1. CLick on "Apps" tab in the Discovery Enviornment and search for "Rstudio-DESeq2".
- 2. Click on the three dots button near the top-right corner of the app and click on "Quick Launch".



3. Click on the "RNAseq_Webinar_DESEq2" quick launch and click on the play button to launch.



- 4. Under "Analysis Name" leave the defaults or make any desired notes.
- 5. Under "Select input files and folder" add the "feature_counts.txt" file we got from our previous RMTA run.

Note: The quick launch feature comes pre-loaded with DESeq2 R script.

- 6. Under "Resource Requirements" request resources as needed or leave for defaults.
- 7. Click **Launch Analysis**. You will receive a notification that the job has been submitted and running with the "Access your running analysis here".
- 8. Clicking on the "Access your running analysis" will open the Rstudio-DESeq2 in another tab in the browser after a brief building phase.

Note: You will be asked to authenticate again to the Rstudio with your CyVerse username and password

- 9. The username and password for logging into Rstudio are "rstudio".
- 10. Finally, once you finish analysis, navigate to the DE tab, select the Analysis window and select the analysis, click "save and complete analysis". Upon clicking complete analysis, the analysis will be completed and all the outputs will be brought back to the analysis folder.

Note: To use our example script, navigate to *Community Data* > *iplantcollaborative > example_data > RNAseq_Webinar *

4.1 Additional information, help

- Link to Webinar Prezi
- See detailed RMTA wiki
- See beginner's guide to DESeq2
- Search for an answer: CyVerse Learning Center or CyVerse Wiki
- Contact CyVerse support by clicking the intercom button on the page.

Fix or improve this documentation

• On Github: Repo link

• Send feedback: Tutorials@CyVerse.org

